

EMBRYOLOGICAL AND CYTOLOGICAL INVESTIGATIONS IN *HYPODISCUS* *ARISTATUS* NEES (RESTIONACEAE)

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(With Plates III and IV)

ABSTRACT

1. *Hypodiscus aristatus* has an ovule which arises as an anatropous initial and later becomes an orthotropous ovule by fusion with the carpel. This could be called a secondary orthotropous condition.

2. *Hypodiscus aristatus* has a monosporic embryo sac of the Polygonum type with 3 non-dividing antipodals and a nuclear type of endosperm.

3. The great majority of mature pollen grains have 3-nuclei. The tube nucleus is deficient in D.N.A.

4. It was found that the diploid chromosome number is $32 + 2$ satellites. Idiograms of chromosome sets for both male and female plants show perfect similarity. Chromosome counts in meiotic metaphase I were in accord with the above finding.

5. Adventitious roots of both sexes have a many-layered pericycle.

6. The wall of the anther has 4 layers. Its opening mechanism is similar to that in the genus *Lilium*.

INTRODUCTION

The family Restionaceae has long attracted attention because of its geographical distribution, its morphological features (evidently transitional between the Liliiflorae, Glumiflorae and Cyperales), and its anatomical structure. The anatomy of the family was treated in detail by Solereder and Meyer¹⁷. Its taxonomy has been the subject of a monograph by Pillans^{13,14}, and more recently Adamson and Salter have discussed the family in their *Flora of the Cape Peninsula*¹. However, the cytological and embryological investigation of the family has not kept pace with these other studies. To the best of the present author's knowledge there is only his own and his student co-workers' short paper on *Restio Dodii* and *Elegia racemosa*², and his short preliminary report in Polish on *Hypodiscus aristatus*⁸. The present paper is a further modest attempt to fill the gap in our knowledge of the cytology and embryology of Restionaceae.

Hypodiscus aristatus was chosen for the investigation chiefly because it differs sharply from other genera and species of this family in its fruits, unbranched stems, its general shape and the marked difference between male and female inflorescences.

TECHNIQUE

The material was collected on the slopes of Table Mountain and its associated ranges near Cape Town in September 1955 and 1956, from such localities as Steenberg plateau, Muizenberg plateau, Camps Bay and Wynberg plateau. The root tips were cut and put into the fixative immediately, at the place of collection. Stems with inflorescences were brought back to the Department of Botany at the University of Cape Town and dissected and fixed there. The following fixatives were used: Navashin fluid, F.A.A., acetic alcohol. The older ovaries were treated after fixation with 20% hydrofluoric acid for 3 days to soften them. After this treatment, and before dehydration and embedding, the material was washed for 24 hours in flowing water. In spite of this precaution it was found that staining with the Feulgen method and with crystal violet was somewhat affected. The following stains were used: Crystal violet after Newton, iron haematoxylin with fast green, safranin with fast green, acetocarmine, and the Feulgen method. In the Feulgen method hot hydrolysis with 1 N HCl was compared with cold hydrolysis with 5 N HCl. The latter was found very satisfactory. The optimum time of hydrolysis in the case of the ovaries of *Hypodiscus aristatus* was 70 minutes.

When working at the Botany Department, University of the Witwatersrand, Johannesburg, a good research microscope with a fluorite oil immersion lens was used. Afterwards at the Poznań University, Poland, a research microscope with two kinds of phase contrast equipment and a condenser was used. The latter was found to be a very practical and convenient research instrument. A camera lucida and new tubular apparatus for drawings were used.

OVULE DEVELOPMENT

The Restionaceae are placed by many authorities on taxonomy, e.g. Warming¹⁸, Wettstein²¹, Engler⁴, Hutchinson⁶, in the order Enantioblastae chiefly on the grounds of their orthotropous ovule. This type of ovule distinguishes the Restionaceae from the very similar Juncaceae (order Liliiflorae or Glumiflorae), or even Cyperaceae, which have anatropous ovules.

In the ontogeny of the ovule in such a family as the Liliaceae or the Gramineae (Andropogoneae) the initial of the ovule starts as a straight continuation of the funiculus. It starts so to say, with the orthotropous stage. At a much later stage, corresponding to the tetrad stage in the nucellus in some Andropogoneae, its upper part starts to bend, one side approaches the funiculus closely and finally it becomes anatropous.

In *Hypodiscus aristatus* however, the earliest initial of the ovule is always anatropous.

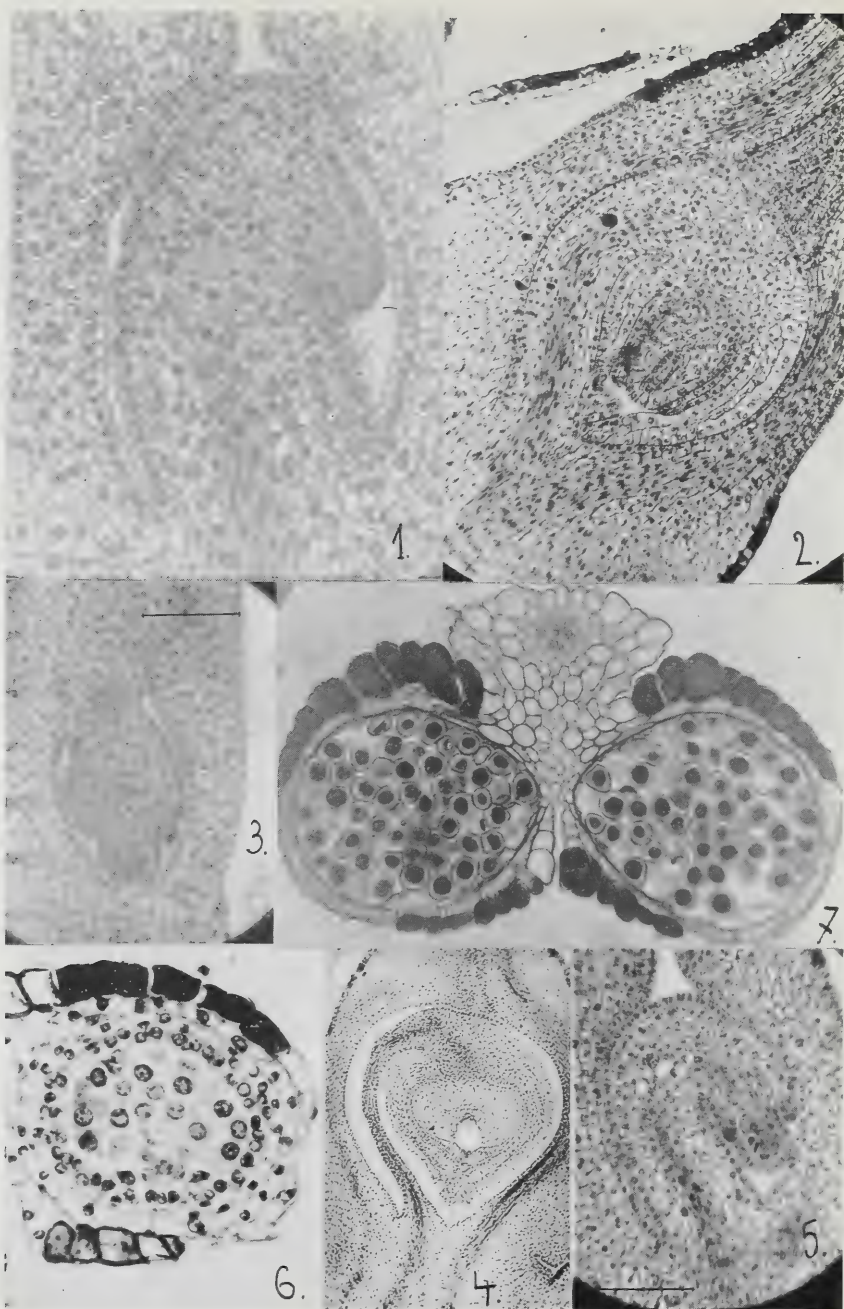


PLATE III

This initial is represented by the small bent down portion at the top of the funiculus. It consists of the nucellus only, preceding any internal differentiation in it and preceding integument initiation (Plate III, Fig. 1). The funiculus as seen in median longitudinal section is an unexpectedly large organ. It is many layers of cells thick. It arises at the bottom of the ovary, when the latter already consists of two carpels and a style. At this stage the funiculus and the initial of the ovule have a distinct epidermis. They are sometimes, even at a more advanced stage, completely free of the carpels (Plate IV, Fig. 16). A narrow slit can be seen between the funiculus, bent initial of the ovule and the inner epidermis of the carpels. At this early stage the inner epidermis lines the whole cavity of the ovary. It makes the line of separation between the funiculus, the top of the bend, the ovule and the carpels, easy to distinguish (Plate III, Fig. 5).

The funiculus is a very peculiarly-shaped, broad organ. In transverse section it is a rather flat organ, crossing the chamber of the ovary from one wall to the other. It is tallest and thickest in the part where it initiates the ovule.

The author still has some hesitation in offering an interpretation of this structure. It might be called the third carpel, but two of its features are against this: (1) it arises much later than the two carpels forming the

PLATE III

FIG. 1.—Slide 10, thickness $8\ \mu$, obj. 45, oc. 10, magnif. after enlargement of photo ± 375 .

The initial of the ovule without integuments. The ovule and the filament are free of carpels. Anatropous condition. The earliest observed stage.

FIG. 2.—Slide 8, 2/1, thickness $8\ \mu$, taken with obj. 10, oc. 15, enlargement about 200.

Older stage of the ovule. Line of separation very clear, but the beginning of the fusion on the top of the bend can be seen.

FIG. 3.—Slide 1^b, thickness $10\ \mu$, taken with obj. 45, oc. 5, magnif. after enlargement about 250.

Older stage of the ovule; both integuments are present. The ovule is fused on the top with the carpel. Orthotropous condition.

FIG. 4.—Slide 160, 2/2 thickness $12\ \mu$, ph. centr. obj. 10, oc. 10, magnif. about 85.

The ovule is mature and fused at the top, in its chalazal part, with one carpel. The stage—mature embr. sac. The vasc. trace supply only through the funiculus—a reminder of the early anatropous stage.

FIG. 5.—Slide 2^c, 2/7, taken with obj. 45, oc. 5 enlargement about 250.

The funiculus and the ovule are still free of the carpels but touch them. The line of separation is clear.

FIG. 6.—Slide 24^b, taken with obj. 45, oc. 10, enlargement about 500.

Pollen grain mother cells in young anther. The anther wall shows 4 layers.

FIG. 7.—Slide A₃, taken with obj. 10, oc. 15, magnif. after enlargement about 200.

An advanced stage of the anther wall; spiralisations in endothecium, tannins in the remains of epidermis, connective.

ovary and internally to them; (2) in the majority of plants it fuses completely with the wall of the ovary in the later stages. On the other hand it could be considered a funiculus, but the following features are against it: (1) it is very thick in median longitudinal section, unlike the typical funiculus, and (2) it is very broad, stretching from wall to wall across the whole chamber. In view of the above hesitation, and since one is compelled to name it somehow, temporarily, it shall be provisionally referred to as a funiculus.

The first fusion occurs at the top of the funiculus bend (Plate III, Fig. 2). The part which first touches the carpel is the highest point of the funiculus and of the ovule initial. It more or less corresponds to the chalaza of the ovules or a little above the chalaza. Contact is soon made along the whole length of the funiculus, the ovule facing the internal epidermis of the carpels. Due to two epidermal layers, one on the funiculus, the other on the inner side of the carpel, the line of separation is visible even at the stage when two integuments are present and the micropyle is formed (Plate III, Fig. 2). Here two further behaviour peculiarities of the funiculus and of the ovule may be noticed: (1) the funiculus can more or less completely fuse with the carpels. Advanced fusion is shown in Plate III, Fig. 3. Thus the ovule achieves the orthotropous stage; (2) or the funiculus may be left free of the carpel even after fusion of the ovule with the carpel has occurred (Fig. 1).

Fusion of the ovule with the carpel may be achieved in two ways: (1) either it is a simple fusion with one carpel only as in Plate III, Fig. 3, or (2) it is a fusion with both carpels as in Fig. 2. One can see from Plate III, Fig. 4 that the funiculus can serve as a partition wall in the cavity of the ovary dividing it into two unequal chambers. This occurred in a minority of cases. In the majority of plants the funiculus fuses more or less completely with a carpel when the ovule matures, thus leaving the ovary one-chambered. The above fact that the ovary can have either one or sometimes two chambers has been long appreciated by monographists of the family (R. S. Adamson, N. S. Pillans), but the nature of this partition wall was not recognised.

The importance of vascular bundle traces in ovules for taxonomic and morphological considerations was recognised by many scientists, e.g. Warming¹⁹, Netolitzky¹¹. In this respect one may find two conditions in *H. aristatus*. In one of these the procambial bundle enters the funiculus and passes through it up to the chalaza of the ovule. This may be found in the ovaries, in which the funiculus functions as a partition wall between two chambers (Plate III, Fig. 4, note the ovule here is anatropous). Or it may as frequently be found in the funiculus which has fused with the carpel. Sometimes even at a very early stage of the ovule, as in Plate III, Fig. 5, one may find the procambial bundle entering through the funiculus and passing

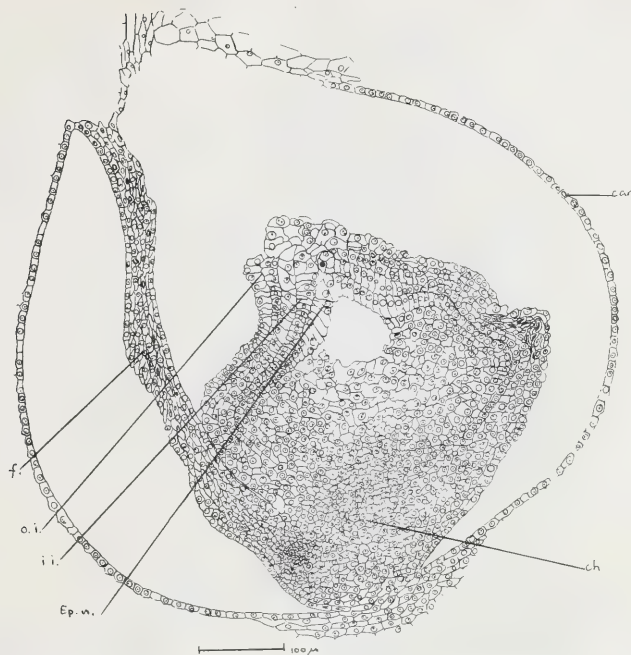


FIG. 1.—Slide 31 2/4, thickness $10\ \mu$, fix. F.A.A., stain: I—Hemat. + Fast green, obj. 10, ocular 15, magnif. 200.

Ovule with mature em. sac. Funiculus forming partition wall between 2 chambers. Fusion with the carpel.

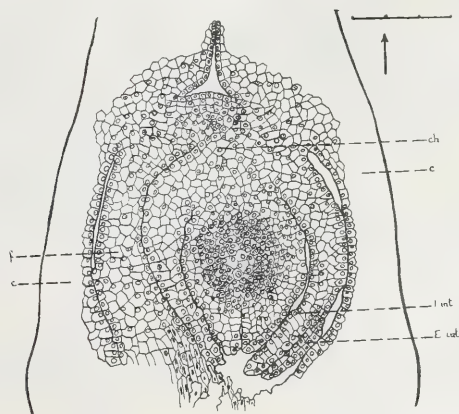


FIG. 2.—Slide 30, 1/3, thickness $10\ \mu$, fix. F.A.A., stain: I—Hemat. + Fast green, obj. 10, oc. 15. Magnif. 200.

Ovule in orthotropous condition, fused with 2 carpels.

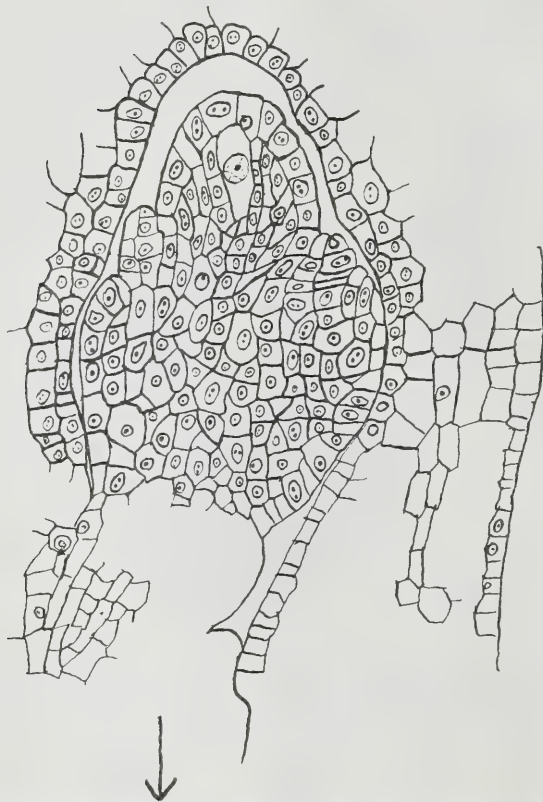


FIG. 3.—Slide 3a, 2/3, thickness 10 μ , fix. Navashin, stain: I—Hemat. + Fast green, obj. 45. oc. 5.
Macrospore mother cell.

upwards to the bend, finally towards the chalaza, as is typical for an anatropous ovule. This first kind of bundle supply to the ovule is certainly common in anatropous ovules but there are ovaries with the second condition, in which the bundle supply enters the ovule at the place of fusion of the chalaza with the carpel.

The ovule in *H. aristatus* is bitegmic and crassinucellate. The nucellus develops a very conspicuous epidermis (Fig. 12) with the cells anticlinally elongated. The cells of this epidermis are longest and biggest at the micropyle.

MACROSPOROGENESIS, EMBRYO SAC DEVELOPMENT AND FERTILIZATION

The initiation of the macrospore mother cell starts either in the layer just beneath the nucellar epidermis or in the next layer. In the ovule at this stage of development, the inner integument just reaches half the height of the nucellus and the outer integument 4-5 layers of cells beneath it has the appearance of a small protuberance. The epidermis of the nucellus still at a stage before its conspicuous differentiation differs slightly from the nucellus core. The formation of a parietal cell from an archesporial one was never observed with certainty. (Fig. 3).

Meiosis soon follows and dyads are formed when the integuments approach the top of the nucellus without as yet covering it (Fig. 4). The epidermal cells begin to elongate. Here and there some anticlinal and even periclinal divisions occur among them. Deviations from normal dyad and tetrad development were never observed. Tetrads are perfectly normal (Fig. 5).



FIG. 4.—Slide 78, 1/8, thickness 12 μ , fix. F.A.A., stain: I—Hemat. + Fast green, imm. lens 98, oc. 10, magnif. 1400.
Dyads.

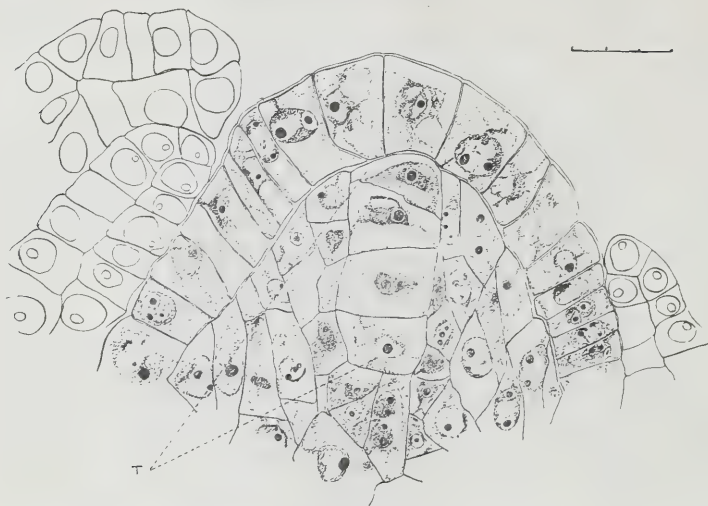


FIG. 5.—Slide 71, 2/4, thickness $12\ \mu$, fix. F.A.A., stain: I—Hemat. + Fast green
f. imm. lens 98, oc. 10, magnif. 1400.
Tetrads.

The uninucleate or 1-nucleate embryo sac appears when the micropyle is nearly formed (Fig. 6). At this time the epidermal cells of the nucellus are elongating. Some of them still divide periclinally. Their nuclei are certainly bigger than the nuclei of nucellus cells. The embryo sac with two nuclei is a bit larger than the 1-nucleate stage. It has a large vacuole between the nuclei (Fig. 7).

The 4-nucleate embryo sac occurs when the micropyle is already formed (Fig. 8). The embryo sac cell is twice as large as in the 2-nucleate stage and the nuclei are perhaps the smallest encountered during the whole course of development of the embryo sac. It is worth noting that the outer integument is outgrowing the inner integument. In this species the sequence of integument development is as follows: the inner integument starts to develop first; the outer integument starts later but develops more quickly, so that in the 2-nucleate embryo sac they are both already approximately of equal height. At the stage of the 4-nucleate embryo sac the outer integument is taller. The 8-nucleate free nuclei stage is very rare. It is common knowledge that this stage is very short lasting. Here the nuclei of the embryo sac reach their smallest size. From this stage on they increase with the further growth of embryo sac and its differentiation. It was considered lucky to obtain one distinct slide of this stage out of about 260 slides of the embryo sac prepared (Fig. 9).



FIG. 6.—Fl. imm. lens 98, oc. 10, magnif. 1400.
1-nucleate embryo sac.

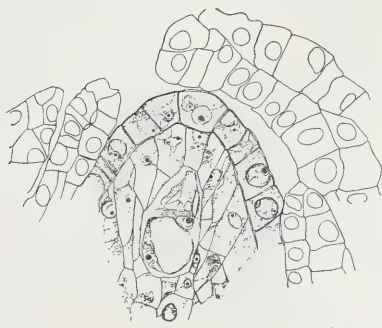


FIG. 7.—Slide 65, $3/2$ thickness $12\ \mu$, fix. F.A.A., H. Fl. 3 days, stain: crystal violet,
fl. imm. lens 98, oc. 10, magnif. 1400.
2-nucleate em. sac.

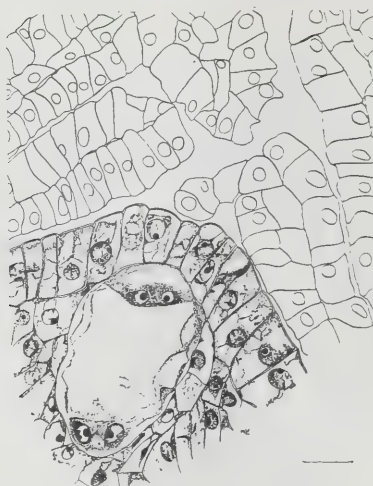


FIG. 8.—Slide 48, 1/last, thickness $8\ \mu$, fix. F.A.A., H. Fl. ac. 3 days, stain: I—Hemat. + Fast green, fl. imm. lens 98, oc. 10, magnif. 1400, compound drawing. 4-nucleate em. sac.

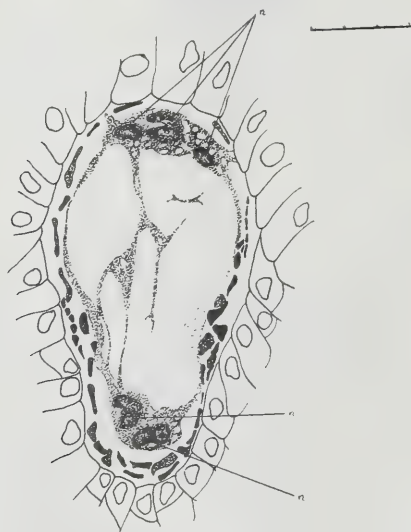


FIG. 9.—Slide 117, 1/6-7, thickness $12\ \mu$, fix. Nav., stain: Feulgen, Ph. oil imm. lens 100, oc. 10, magnif. 1300. 8 free nuclei stage in em. sac.

The mature embryo sac has a typical egg apparatus: egg cell and two synergids (Fig. 10). In the majority of the observed embryo sacs the big vacuole of the egg cells is above the nucleus and in the synergids is below it. The synergids when first formed often look like those in Fig. 10.

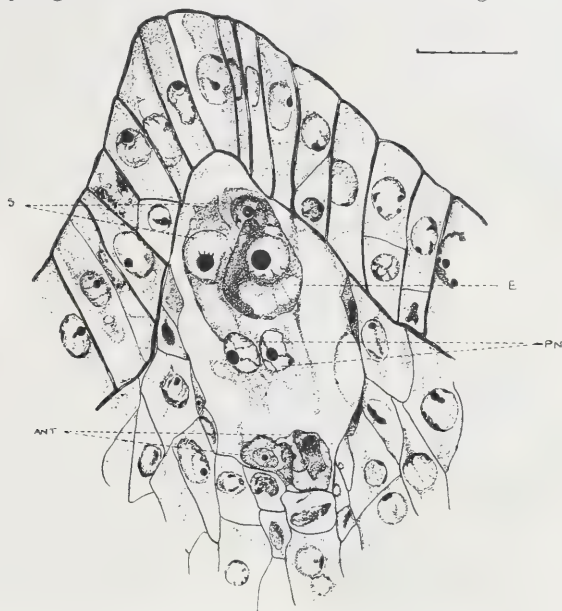


FIG. 10.—Slide 61b, 3/1, 3/2, thickness $15\ \mu$, fix. Nav., H. Fl. ac. 3 days, stain: I—Hemat. + Fast Green, fl. imm. lens 98, oc. 10, magnif. 1400.
Mature em. sac.

Later, with the increasing age of the embryo sac, the synergids develop hooks. In some embryo sacs which were apparently overmatured (without fertilization), but before their degeneration, the synergids had distinct hooks and a filiform apparatus.

The polar nuclei are suspended in the cytoplasm of the embryo sac very close to the egg apparatus. The three antipodals persist, sometimes even until the moment of fertilization of the secondary nucleus. Sometimes they begin degenerating before this stage. The antipodals, contrary to our findings in *Restio Doddii*, and in *Elegia racemosa* (Borwein *et al.*²), never divide.

The polar nuclei fuse late. They are often seen to be lying in contact when the male nucleus approaches them. In median section, the mature embryo sac before fertilization has the shape of an octagon

with the micropyle-chalaza axis being much longer than the axis perpendicular to it. It increases in size considerably during the maturation period. The epidermis of the nucellus now reaches full development. Some lignification of the epidermal walls appears during the time of syngamy. It follows from the above findings that the embryo sac in *H. aristatus* is monosporic of the Polygonum type.

The most outstanding feature of the mature embryo sac is that fertilization in it occurs very seldom. A very large proportion of the embryo sacs degenerate without fertilization. In those fertilized the remains of the pollen grain tube in the micropyle are very soon resorbed. Embryo sacs at the 4-nucleate stage of the endosperm, and without any pollen grain tube remains in the tissue, are very frequently encountered. Unfortunately no clear picture of the sperms on their way to the female gametes

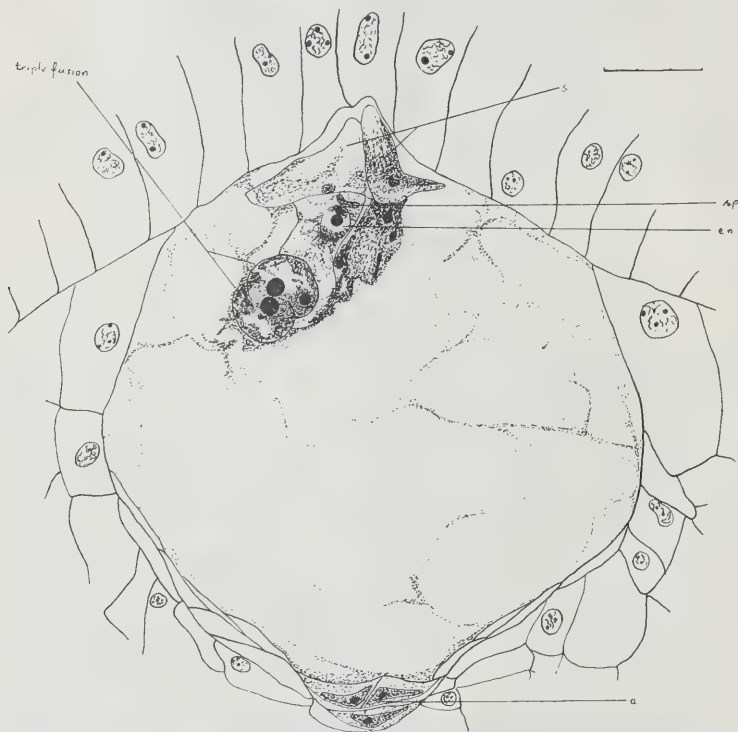


FIG. 11.—Slide 219, 2/1-4, thickness 12 μ , fix. Nav., stain: crystal violet, Ph. oil imm. 100, oc. 8, magnif. 1600, compound drawing.
Double fertilization in em. sac.

was found. Usually there were stages of late syngamy with many endosperm nuclei in the embryo sac cytoplasm. Fig. 11 represents the earliest fertilization stage found.

The polar nuclei are here in advanced fusion with the male nucleus. The second sperm (Fig. 11—sp.) is just touching the egg cell nucleus. The synergids display their hooks. At the bottom of the embryo sac the already reduced but still distinct antipodals may be seen. In this material it is rather an early stage. In the slides, stages of delayed syngamy in the egg cell and advanced development of endosperm prevailed. The proembryo stage was never seen in the course of the present investigation. Even the mature zygote (Fig. 12) was rare. The one in Fig. 12 also

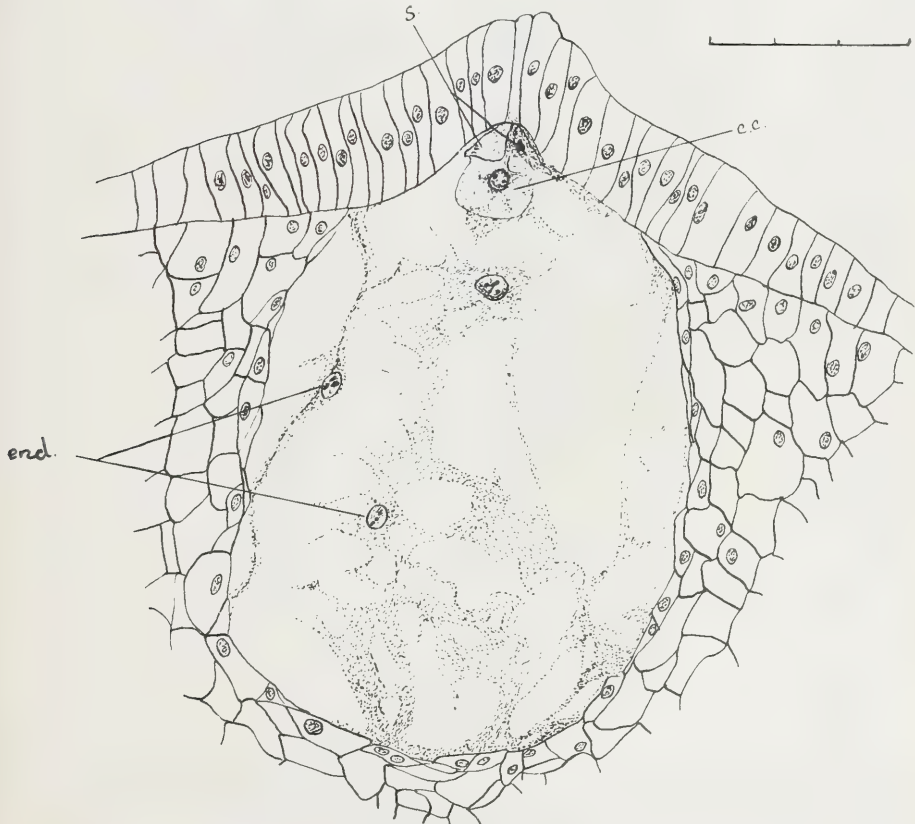


FIG. 12.—Slide 206, 2/3—2/5, fix. F.A.A., stain: Feulgen + L. green, Ph. obj. 20, oc. 8, magnif. 375, compound drawing.

Young zygote, endosperm nuclei. ec—young zygote

seemed exceptional, because of the fact that syngamy was accomplished when only three nuclei of the endosperm were formed. The most frequent stage found (Fig. 13) was that of belated syngamy in the egg and in many nuclei of the endosperm.

In one embryo sac 16 free nuclei were found in the endosperm and in the egg cell the fusion of the male nucleus with the female one was still not accomplished. It was certainly not a matter of chance only but a very significant feature and probably relevant to the tendency to produce some fruits with undeveloped embryos.

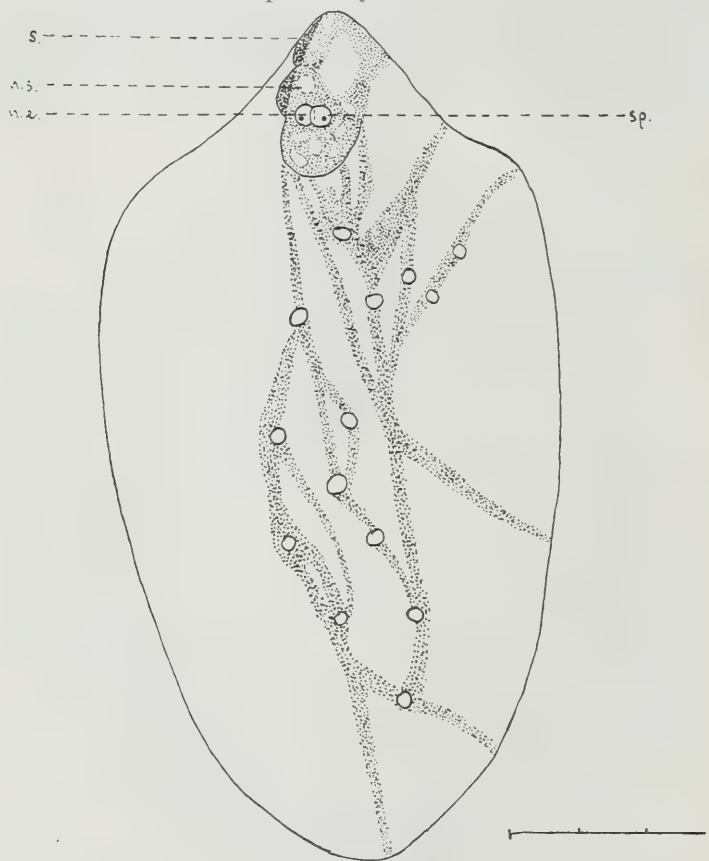


FIG. 13.—Slide 150, 2/1-3, thickness $14\ \mu$, fix. Nav., stain: Feulgen + L-green, obj. 40, oc. 12, magnif. 600, compound drawing, schematically.

Belated syngamy in egg, 14 endosperm nuclei.

s.—synergid, n.s.—synergid nucleus, n.e.—egg. nucleus, sp.—sperm.

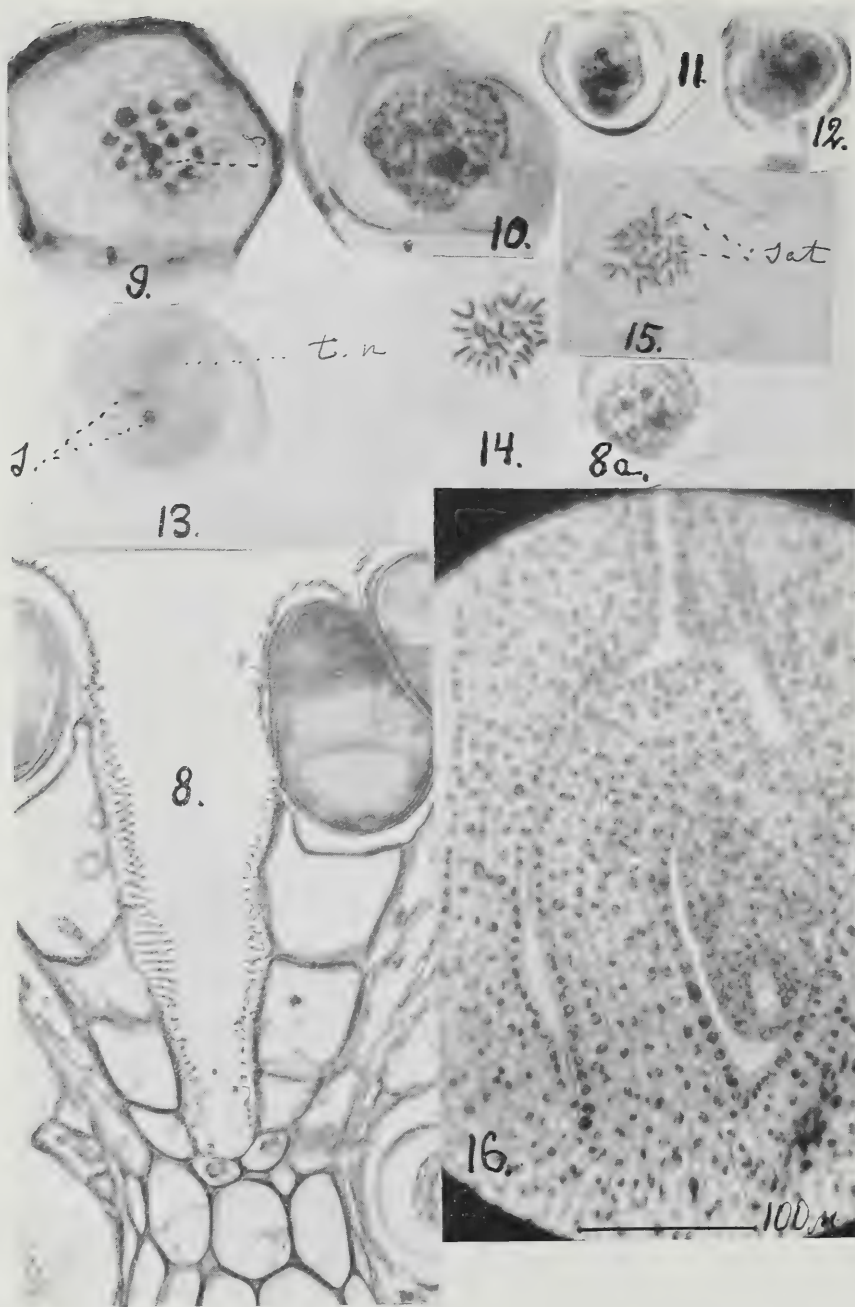


PLATE IV

THE ANTHER STRUCTURE, POLLEN GRAIN DEVELOPMENT AND MEIOTIC CHROMOSOME NUMBERS

All members of the Restionaceae are dioecious. The male floret of *Hypodiscus aristatus* has six scales in the perianth and three stamens. The anthers here have only two loculi (pollen grain sacs). The structure of the anther wall and the relationship of the anther to the filament are not yet described. The youngest stage encountered in this investigation corresponds to the early meiotic prophase in pollen grain mother cells (Plate III, Fig. 6). Here, the anther wall is built of four layers. The epidermis, still very distinct, starts to store some tannins. The next layer, the endothecium, is still very young, containing cytoplasm and nuclei. The transitional layer is already slightly depressed. The most internal layer, the tapetum, shows the cells in full vigour, filled with the cytoplasm and nuclei. Some of the tapetal cells are already binucleated.

PLATE IV

FIG. 8.—Slide A, thickness 10 μ , taken with Bausch & Lomb fluorite imm. 98, oc. 10, magnif. about 1000.

Part of the anther, with the thinnest portion of the wall shown, where opening occurs. Hair-like cutinisation of the epidermis. Connective. Mature pollen grain.

FIG. 8^a, one mature pollen grain from the above photo enlarged and separated to show 4 nuclei in it.

FIG. 9.—Slide 22^b, 4/4, fix. F.A.A., stain: pur. crystal violet, thickness 12 μ , taken with Bausch & Lomb fluorite oil imm. 98, oc. 10, magnif. 1000.

Pollen grain mother cell, meiotic metaphase I, $n = 16 + 1$ satellite.

FIG. 10.—Slide A, stain: I—Hemat. + Fast green, taken with fluorite oil imm. 98, oc. 10. Yellow filter No. 15, exposure 6 in. magnif. about 1000.

Three-nucleate pollen grain.

FIG. 11.—Slide 17, stain: I—Hemat. + Fast green, obj. 60, oc. 10, magnif. about 600.

Four-nucleate pollen grain.

FIG. 12.—As above.

Five-nucleate pollen grain.

FIG. 13.—Slide 30 E₄, left mark, lower row, fix. Ac. Alc., stain: Feulgen, fluorite oil imm. 98, oc. 10, exposure 18 in., high contrasted filter, magnif. about 1100.

S—sperm nuclei.

T.N.—unstained tube nucleus.

FIG. 14.—Slide D₄, 3/2, at 3^h, 8th cell f. epiderm. stain: pur. crystal violet, taken with fluorite oil imm. 98, Bausch & Lomb, oc. 10, exposure 9 in., filters—green and ground glass, magnif. about 1300.

Root tips from the male plant. $2n = 32$.

FIG. 15.—Slide D., 2/7, at 10^h, 5th cell from epiderm., taken with Bausch & Lomb fluorite oil imm. 98, oc. 10, exposure 9 in., 2 filters, magnif. 1100, as above. Both satellites are distinct.

Sat—satellites.

FIG. 16, slide 4, 1/3, B & L, obj. 45, oc. 5, magnif. 250.

Orthotopous condition of the ovule in an even later stage, when the micropyle is formed and beginning of the embr. sac initiated.

At the stage of dyads and tetrads the anther wall shows distinct tapetal cells in the process of absorption, the remains of transitional layers, the endothecium or mechanical layer (not yet fully developed) and the epidermis, already charged heavily with tannins. The tannins here are so persistent that even a week's treatment in Stockwell solution, or in 1% Chromic acid, does not wash them out at all.



FIG. 15.—Slide 6, penultimate section in 2nd row, thickness 12 μ , fix. F.A.A., stain: I—Hemato + Fast green, obj. 15, oc. 10, photo enlargement 150.
Anther free of filament. Dyads, tetrads.

Fig. 15 represents a cross section through the upper part of the anther and the filament. The filament here has one trace and it is free of the anther. At a lower level the filament is fused completely to the connective and to the anther (Plate III, Fig. 7).

At the stage represented in Plate III, Fig. 7, the cells of the tapetum have already disappeared leaving only a line of grains. The epidermis has increased in cell size. The biggest change has occurred in the endothecium. Its cells have developed spirals stained distinctly with safranin. They represent the opening mechanism of the wall. This structure of the wall reminds one of the *Lilium* anther structure. Fig. 14 illustrates the mature and opened anther. One can see that wall breakage occurs in its inner corner, again similarly to the genus *Lilium*. This part of the wall has a complex of very thin cells in the endothecium and in the epidermis. This is the place of the smallest resistance to tension and adapted to easy breakage. In the same area one can see under higher magnification (Plate IV, Fig. 8a) that epidermal cells reveal a peculiar ornamental structure—cutinised teeth on the outer wall.

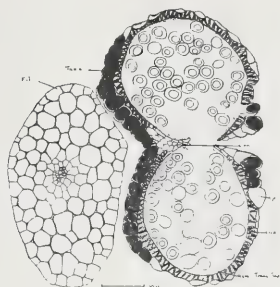


FIG. 14.—Slide A5, 2nd row, thickness $20\ \mu$, fix. F.A.A., stain: Safr. + Fast green, obj. 10, oc. 15, magnif. 200–220. 50–55.
Mature anther. Opening.
Mature endothecium.

One finds therefore two non-cellulosic structures in the anther wall of *Hypodiscus aristatus*: (a) the lignified spirals of the mature endothecium, (b) cutinised teeth of the epidermis.

To summarise the findings of this section: The young anther has a 4-layered wall. The mature anther has an endothecium with spirals and opens at a distinctive thin region at the inner corner (as in the genus *Lilium*, but not as in *Gramineae*).

The cytokinesis in the pollen grain mother cells is of the successive type. There are no abnormalities in formation of dyads and tetrads. The meiotic metaphase I was often observed. It comes out especially well after Newton's crystal violet stain. It shows 16 bivalents and one satellite (Plate IV, Fig. 9). Few metaphases have $n = 15$. The prevailing number was certainly 16.

The morphology of the pollen grains of some members of the Restionaceae has already been described by Erdtmann⁵. The mature pollen grain is loaded with starch. After staining with iron-hematoxylin or aceto-carmin it has one large tube nucleus and two very small male nuclei, the so-called sperms (Plate IV, Figs. 10 and 13). The size of these male nuclei measured in the five best pollen grains, which had been stained by the Feulgen method, was: width—minimum $0.8\ \mu$, maximum— $1.6\ \mu$, length—minimum $3.0\ \mu$, maximum— $4.5\ \mu$. With the technique used in the present work it was impossible to reveal any trace of male cytoplasm around them. The great majority (probably 85%) of slides showed 3 nuclei in the pollen grains.

Hypodiscus aristatus in this respect is very similar to the Gramineae and Juncaceae in which the 3-nucleate condition is typical for the family. Besides the prevailing 3-nucleate condition in *H. aristatus* some pollen grains with 2 nuclei occur and exceptionally some with 4 nuclei (Plate IV, Fig. 11 and 8a) and even, rarely, with 5 nuclei (Plate IV, Fig. 12).

In the slides from material fixed in acetic-alcohol and stained by the Feulgen method, the mature pollen grain shows two sperms stained well with Feulgen and a completely unstained tube nucleus. To check findings, some sections of the same anther were divided between two slides; one was stained with iron-haematoxylin, another by the Feulgen method. In the first one three nuclei were stained: the tube nucleus and two sperms. The second slide shows only two sperms stained red and the tube nucleus unstained. Comparing these results obtained after different staining, one can only come to the following conclusion: the tube nucleus in the mature pollen grain of *H. aristatus* is completely deprived of D.N.A., and therefore it is not stained by the Feulgen method. But it has a lot of nucleoproteins and that is why it is stained with iron-haematoxylin and aceto-carmin. D.N.A. deficiency in the tube nucleus is generally not a new finding, but it is a new fact for the Restionaceae. The above observations were based on the material collected in 1950 only. Then I asked my botanist colleague in Cape Town, Miss du Plessis, to collect and fix in acetic alcohol fresh material in July–August 1956. Slides prepared from this fresh material, and stained by the Feulgen method, completely confirmed the first conclusion: the small sperm nuclei in mature pollen grains have D.N.A. and the big tube nucleus lacks it completely (Plate IV, Fig. 13).

It is certain now that deficiency in D.N.A. in the tube nucleus is not dependent on the season (year) of collection.

La Cour⁹, reported such instances some years ago, but for tube nuclei in pollen grain tubes. Bryant³, recently opposed this interpretation of tube nuclei in *Tradescantia*.

Present findings evidently support La Cour's observations. In view of these findings of D.N.A. deficiency in the tube nucleus, one can now interpret the origin of the supernumerary nuclei in pollen grains, namely those with 4 and 5 nuclei. The supernumerary nuclei were all found after staining with iron haematoxylin and aceto-carmin. To date no supernumerary nuclei have been found in the slides stained with the Feulgen method. It proves that supernumerary nuclei have the same nature as the tube nuclei. In view of this, and until such should be found with Feulgen staining, it is safer to assume that the supernumerary nuclei are the products of division of the tube nucleus or the results of its degenerate fragmentation.



FIG. 16.—Reichert's fluorite imm. lens 100, oc. 17, magnif. 2600.
Idiogram of chromosome set of the female plant.



FIG. 17.—Optics as above.
Idiogram of chromosome set of the male plant.

DIPLOID CHROMOSOME NUMBER, IDIOGRAMS OF CHROMOSOMES AND SOME ANATOMICAL PECULIARITIES OF ADVENTITIOUS ROOT STRUCTURE

Investigation of root tips of adventitious roots was as usual a good source of somatic metaphases. First it was found that the diploid number of chromosomes was 32 in the roots of male plants. There were two satellites (Plate IV, 14 and 15 and Fi. 19). Contrary to our first expectation (Krupko, 1957), there was no difference between the two SAT-chromosomes. Comparing many metaphases of both male and female plants the morphological equality of both SAT-chromosomes was established. There was slight variation in weakly marked bending of the chromosomes in both sexes (Figs. 18 and 19).

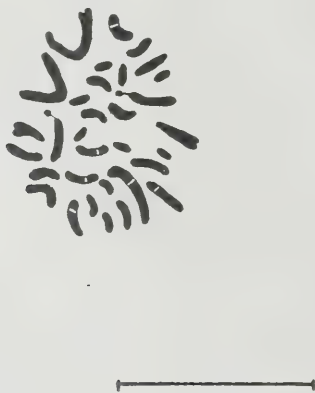


FIG. 18.—Slide A, 3/8, thickness 8 μ , fix. Navashin, stain: I—Hemat., optics as Fig. 19.

Female plant. Metaphase from root tip. Two satellites.

An idiogram was drawn for the male chromosome set from ten selected metaphases. One can easily distinguish amongst them: 2 V-shaped pairs, 1 SAT pair, 1 L-shaped pair, 4 pairs slightly curved in the middle, 8 rod-shaped pairs of different lengths. An idiogram of the chromosomes of the female plant was drawn in the same way (Fig. 16). Complete similarity in chromosome sets of both sexes was found. The female plant chromosomes have the same diploid number = 32, and the same chromosome morphology as the male plant. Such a perfect similarity



FIG. 19.—Slide D3, 1/7, thickness $10\ \mu$, fix. Navashin, stain: pur. Cr. Viol., Reichert's fluorite oil imm. lens 100, oc. 17, magnif. 2600.
Male plant. Metaphase from root tip. Two satellites.

was rather unexpected because the male plants differ from female ones in shape of the inflorescences. The only minute difference one may find is shown by the female plant: its chromosomes are perhaps slightly wider.

On cutting more mature parts of the same roots in the male and female plants it was found that the pericycle here is formed of many cell layers, usually 6–8 (Fig. 20).

This feature deserves to be noted and published, because a pericycle of many layers is a distinctive character of Gymnosperms. In Angiosperms it is only found in a few exceptional plants. For example Esau's "Plant Anatomy"^{5a} gives only 6 such exceptional plants. A many-layered pericycle was, however, recorded from some Restionaceae species by Solereder and Meyer "Systematische Anatomie der Monokotyledonen" H. IV, 1929, p. IV. 29. The following species were mentioned as having a large pericycle: *Leptocarpus incurvatus*, *Restio triflorus*, *Thamnochortus fruticosus*, and *Hypodiscus willdenowia*. To that list we can now add *Hypodiscus aristatus*.

Other species of Restionaceae have narrow, 1-layered pericycles, typical for Angiosperms.

DISCUSSION

The value of the type of ovule for taxonomy and evolution was a much discussed subject in the literature. The orthotropous ovule is usually recognised as a primitive character. From this point of view, the Restionaceae have a distinctly primitive feature as compared with the order

Liliiflorae with an anatropous ovule—an advanced character. In this respect *H. aristatus* would differ from other Restionaceae in not having a purely orthotropous ovule but one initially starting from an anatropous stage. This feature places *H. aristatus* much nearer to the Liliiflorae than other members of the family.

Another character in common with the Liliiflorae in *H. aristatus* is the persistence of the antipodals in embryo sac development. In *Restio dodii* and *Elegia racemosa*² the antipodals form a complex of cells similar to that in the Gramineae. In *H. aristatus* the antipodals never divide but are long lasting elements of the embryo sac. The wall of the anther and its opening mechanism are reminiscent of similar structures in many Liliaceae.

Hutchinson's recent opinion⁷ is that Restionaceae should be classified in common with Juncaceae, Thurniaceae, and Centrolepidaceae in the new order, Juncales. Inside this order Restionaceae are the nearest to Juncaceae and both originate from the Liliiflorae. This new order of Juncales in Hutchinson's system is a part of Division III, Glumiflorae together with Cyperales and Graminales. The embryology of Juncales is only a little better known than the embryology of the old order Enantioblastae in which the Restionaceae were usually included in previous systems. From this scarce information it is known the Juncaceae develop 3-nucleated pollen grains in tetrads (Schürhoff¹⁶). *Hypodiscus aristatus* has single and 3-nucleated pollen grains. Thus this species differs from Juncaceae and is rather similar to Gramineae with 3-nucleated single pollen grains present as a rule. The tendency to develop 3 nuclei in mature pollen grains was observed previously in other members of Enantioblastae (Weinzieher²⁰), who reported it long ago in *Xyris indica*; and Palm¹² in *Eriocaulon septangulare*. Perhaps one must be cautious in any assumption based on embryological facts in the Restionaceae, having in mind the small number of species investigated (only three amongst more than three hundred known and described). Embryological knowledge of the order Enantioblastae is no better (see Maheswari¹⁰). Few species have been studied. Perhaps the best known is the genus *Tradescantia*. Embryological studies of Juncaceae have progressed only slightly further. It follows from the above that it is too early to make any conjectures about relationships and the taxonomic position of Restionaceae on the basis of embryological and cytological characters.

With our present knowledge one can only trace the affinities of the three species of Restionaceae studied. *Restio dodii* and *Elegia racemosa* have affinity to the Gramineae in that they develop numerous antipodals, but they differ from Gramineae and Liliiflorae generally in the possession of a true orthotropous ovule. *Hypodiscus aristatus*, displaying an anatropous stage in ovule development shows affinity to the Liliiflorae

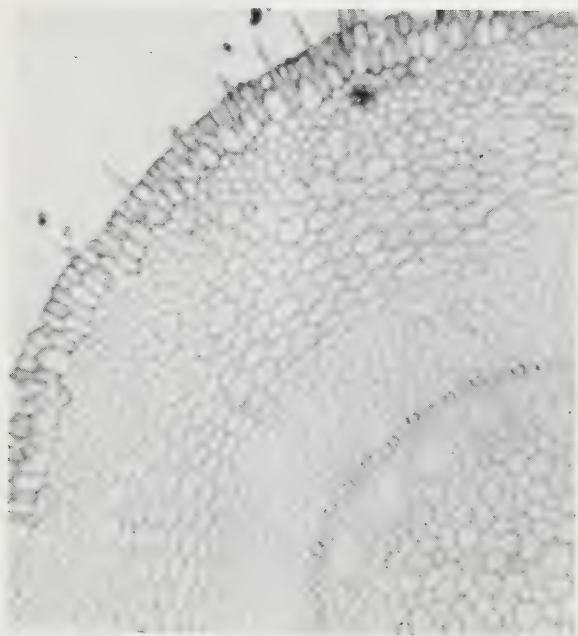


FIG. 20.—Slide NG B7, adv. root, thickness $20\ \mu$, fix. Navashin, stain: Safr. + Fast green, obj. 10, oc. 10, photo enlargement 110.

Male plant. Transect. adv. root showing pericycle many layers thick.

end.—endoderm.

per.—pericycle.

and Juncaceae. Other links with these groups are the three persistent antipodals, the opening mechanism in the anther and the strong 4-layered structure of the anther wall. *H. aristatus* differs from Juncaceae and other Liliiflorae in the structure of the mature pollen grain since a 3-nucleate, single pollen grain is certainly a feature much nearer to Gramineae.

To end this comparison it might be well to recall the primitive character in the root structure of *Hypodiscus aristatus*. It has a pericycle many layers thick, which is a character typical for Gymnosperms, especially for Coniferales. The fact that *H. aristatus* is the fifth species in the family with this character, certainly speaks for the primitive nature of the family.

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